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(54) Title: ZWITTERIONIC COMPOUNDS AND THEIR USE TO CROSS-LINK COLLAGENOUS MATERIALS

(57) Abstract

Cross-linking of collagenous material may be achieved by treatment with compounds of the formula (I) $X_m(R^1)(CHO)_n$, wherein R^1 is an organic group having n+m functionality, X is a zwitterionic pendant group having an overall neutral charge, m is at least 1 and n is at least 2 (or a gem-diol, hemiacetal or acetal thereof). This results in the pendant amine groups of lysine residues present on the surface of the collagenous material to undergo a condensation reaction with the aldehyde groups present in formula (I) to form an imine linkage, thus fixing the biological moieties. The incorporation of the zwitterionic groups, suitably phosphoryl choline derivatives may have significant benefits in extending the life and reducing calcification of collagenous materials. One specific compound is 3-(oxyethyl-2'-(trimethylammoniumethyl)phosphate)-pentan-1,5-dial.

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ZWITTERIONIC COMPOUNDS AND THEIR USE TO CROSS-LINK COLLAGENOUS MATERIALS

The present invention relates to the synthesis of phosphate compounds and to their use to form biocompatible materials, especially to react with proteinaceous materials to cross link and biocompatibilise them.

It is known that compounds having phosphoryl choline groups and other zwitterionic groups, have useful biocompatibilising properties. Coatings of polymers with pendant phosphoryl choline groups have been shown to be useful as coatings to render blood contacting devices non-thrombogenic. Contact lenses formed from hydrogel polymers with pendant PC groups are less subject to protein deposition, lipid deposition and bacterial adhesion than contact lenses having similar water contents.

We have also described a variety of reagents which are useful for derivatising pre-formed surfaces, for instance of polymeric substrates, to introduce PC groups. Such reagents are generally mono functional, that is each reagent molecule includes a single PC group and a single reactive group. Examples of some such reagents are described in EP-A-0157469, EP-A-0515895 and EP-A-0556216. In EP-A-0515895 reagents which are capable of reacting with amino groups at surfaces to give amine linkages are described. In EP-A-0556216 compounds which react with surface amino groups include activated amine groups.

In WO-A-9301221 we described copolymers of ethylenically unsaturated PC group containing monomers and copolymerisable comonomers selected so as to give suitable surface binding characteristics. One class of comonomers includes a reactive group by which covalent bonding to an underlying surface may be carried out. Examples of covalent reactive groups include an aldehyde group.

It has been reported that PC may have significant benefits in extending the life and reducing calcification of tissue valves, although the means by which PC had been incorporated into such heart valve was not disclosed.

Monomers for use in forming condensation polymers, such as polyesters and polyurethanes have been described. For instance in EP-A-0199790 and EP-A-0275293, monomers comprising two hydroxyl groups are used to react with di-isocyanates and dicarboxylic acids respectively to form polyurethanes and polyesters.

Processes for synthesising dialdehyde compounds have been described. For instance ribose derivatives when reacted with sodium periodate are oxidised at the 3 and 4 hydroxyl groups with the C_3-C_4 bond being cleared to generate two aldehyde groups.

In US-A-4203893 (& EP-A-0001197) cytidine diphosphocholine, which comprises a ribose moiety, is periodated and the dialdehyde reaction product reacted with compounds having free primary amine groups, such as polypeptides or proteins comprising lysine moieties or amino polysaccharides. The diphosphocholine moiety is zwitterionic but has an overall anionic charge (it is not neutral).

In EP-A-0140640 inositol phosphate dialdehyde derivative is reacted with an amine containing hemoglobin derivative.

In J.C.S. Perkin II (1976) 1162-1165 Astin, K. et al describe phosphate derivatives of various terpene derivatives, including some cyclic alkenes and acyclic alkadienes, for solvolysis.

Biological tissues can be "fixed" by allowing them to soak for some minutes in a dilute solution of glutaraldehyde. The glutaraldehyde is used to cross link proteins by their amine groups. Lysine residues in the proteins are the most common source of these amine groups. The aldehyde and amine undergo a condensation reaction to form an imine with the subsequent loss of water. This process is described further in Cheung, D. T. and Nimni, M. E., Connective Tissue Research, 10, 187-216 (1982).

The major constituent of bioprosthetic implants is the protein collagen. To increase the stability of such implants against biodegradation and to reduce the antigenicity, collagen is cross-linked with glutaraldehyde. The problem is that, after implantation, calcification onto the implant gradually occurs, rendering it necessary to replace the implant. The present inventors have established that a PC derivative having two aldehyde groups, or aldehyde group precursors, is useful to replace glutaraldehyde in tissue fixing.

The present inventors have synthesised novel functional phosphate diester compounds, which are suitably phosphoryl choline derivatives, which can be used to replace glutaraldehyde to fix tissue and have devised a synthetic method for producing such compounds.

According to the invention there is provided a new process in which a zwitterionic crosslinker of the formula I

$$X_m (R^1) (CHO)_n \qquad I$$

in which X is a zwitterionic pendant group having an overall neutral charge,

R^1 is an organic group having n+m functionality,

m is at least 1 and

n is at least 2,

or a gem-diol, hemiacetal or acetal derivative thereof, is contacted in aqueous solution with a substrate having pendant primary amine groups, under conditions allowing reaction of the CHO groups of the compound of the formula I with the primary amine groups of the substrate.

It is generally preferred for the reaction conditions to be such that all of the aldehyde groups (or acetal derivative thereof) react with primary amine groups. Thus the conditions under which the compound of the formula I is contacted with the substrate having pendant amine groups will include lower than stoichiometric amounts of the compound of the formula I. Alternatively, where, following contact of the compound of the formula I with substrate having pendant amine groups results in a product having residual aldehyde groups, the treated substrate may be subjected to a post-treatment step in which residual aldehyde groups are reacted with amine group containing compounds in solution.

Preferably the compound of the formula I is used to replace glutaraldehyde in a tissue fixing process. Thus the proteinaceous substrate preferably comprises primarily collagenous material, of which the lysine residues are reacted with the aldehyde groups.

The substrate is preferably proteinaccous, and is most preferably a collagenous substrate. The process of the invention provides crosslinking between pendant amine groups of lysine moieties of the substrate.

The collagenous material may be skin, connective tissue, bone or the organic matter of teeth. Tissues which may usefully be treated in the invention include heart valves tissue and vessels, veins and arteries, ligaments, tendons and Farcia lata, dura matter, pericardium, nerves and cornea implants.

According to the present invention there is also provided crosslinked proteinaceous materials produced by the process of the present invention. The use of

crosslinking compounds incorporating a zwitterionic pendant group has been found to produce crosslinked collagenous products which retain good mechanical properties and are expected to have reduced tendency to calcification.

The group X may be a betaine group, for instance a sulpho-, carboxy- or phospho-betaine. A betaine group must have no overall charge and is preferably therefore a carboxy- or sulpho-betaine. If it is a phosphobetaine the phosphate terminal group must be a diester, ie be esterified with an alcohol. Such groups may be represented by the general formula II

$$-X^3\text{-}R^{24}\text{-}N^+(R^{25})_2\text{-}R^{26}\text{-}V \qquad \text{II}$$

in which X^3 is a valence bond, -O-, -S- or -NH-, preferably -O-;

V is a carboxylate, sulphonate or phosphate (diester-monovalently charged) anion;

R^{24} is a valence bond (together with X^3) or alkylene -C(O)alkylene- or -C(O)NHalkylene preferably alkylene and preferably containing from 1 to 6 carbon atoms in the alkylene chain;

the groups R^{25} are the same or different and each is hydrogen or alkyl of 1 to 4 carbon atoms or the groups R^{25} together with the nitrogen to which they are attached form a heterocyclic ring of 5 to 7 atoms; and

R^{26} is alkylene of 1 to 20, preferably 1 to 10, more preferably 1 to 6 carbon atoms provided that when V is a sulphonate anion, R^{26} is alkylene of 6 or more carbon atoms. One preferred sulphobetaine monomer has the formula III

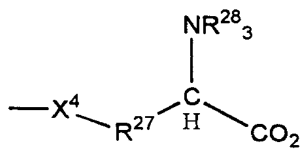
$$\begin{array}{l} R^{16} \\ | \\ -{}^{\oplus}N\text{-}(CH_2)_d\text{-}SO_3^{\ominus} \qquad \text{III} \\ | \\ R^{16} \end{array}$$

where the groups R^{16} are the same or different and each is hydrogen or C_{1-4} alkyl and d is from 2 to 4.

Preferably the groups R^{16} are the same. It is also preferable that at least one of the groups R^{16} is methyl, and more preferable that the groups R^{16} are both methyl.

Preferably d is 2 or 3, more preferably 3.

Alternatively the group X may be an amino acid moiety in which the alpha carbon atom (to which an amine group and the carboxylic acid group are attached) is joined through a linker group to the group R^1. Such groups may be represented by the general formula IV



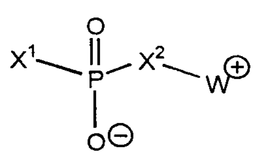
IV

in which X^4 is a valence bond, -O-, -S- or -NH-, preferably -O-,

R^{27} is a valence bond (optionally together with X^4) or alkylene, -C(O)alkylene- or -C(O)NHalkylene, preferably alkylene and preferably containing from 1 to 6 carbon atoms; and

the groups R^{28} are the same or different and each is hydrogen or alkyl of 1 to 4 carbon atoms, preferably methyl, or two of the groups R^{28}, together with the nitrogen to which they are attached, form a heterocyclic ring of from 5 to 7 atoms, or the three group R^{28} together with the nitrogen atom to which they are attached form a fused ring structure containing from 5 to 7 atoms in each ring.

X is preferably of formula V



V

in which the moieties X^1 and X^2, which are the same or different, are -O-, -S-, -NH- or a valence bond, preferably -O-, and W^+ is a group comprising an ammonium, phosphonium or sulphonium cationic group and a group linking the anionic and cationic moieties which is preferably a C_{1-12}-alkylene group.

Preferably W contains as cationic group an ammonium group, more preferably a quaternary ammonium group.

The group W^+ may for example be a group of formula $-W^1-N^+R^{23}{}_3$, $-W^1-P^+R^{23a}{}_3$, $-W^1-S^+R^{23a}{}_2$ or $-W^1-Het^+$ in which:

W^1 is alkylene of 1 or more, preferably 2-6 carbon atoms optionally containing one or more ethylenically unsaturated double or triple bonds, disubstituted-aryl, alkylene aryl, aryl alkylene, or alkylene aryl alkylene, disubstituted cycloalkyl, alkylene cycloalkyl, cycloalkyl alkylene or alkylene cycloalkyl alkylene, which group W^1 optionally contains one or more fluorine substituents and/or one or more functional groups; and

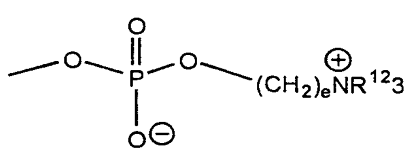
either the groups R^{23} are the same or different and each is hydrogen or alkyl of 1 to 4 carbon atoms, preferably methyl, or aryl, such as phenyl or two of the groups R^{23} together with the nitrogen atom to which they are attached form a heterocyclic ring containing from 5 to 7 atoms or the three groups R^{23} together with the nitrogen atom to which they are attached form a fused ring structure containing from 5 to 7 atoms in each ring, and optionally one or more of the groups R^{23} is substituted by a hydrophilic functional group, and

the groups R^{23a} are the same or different and each is R^{23} or a group OR^{23}, where R^{23} is as defined above; or

Het is an aromatic nitrogen-, phosphorus- or sulphur-, preferably nitrogen-, containing ring, for example pyridine.

Preferably W^1 is a straight-chain alkylene group, most preferably 1,2-ethylene.

Preferred groups X of the formula V are groups of formula VI:



VI

where the groups R^{12} are the same or different and each is hydrogen or C_{1-4} alkyl, and e is from 1 to 4.

Preferably the groups R^{12} are the same. It is also preferable that at least one of the groups R^{12} is methyl, and more preferable that the groups R^{12} are all methyl.

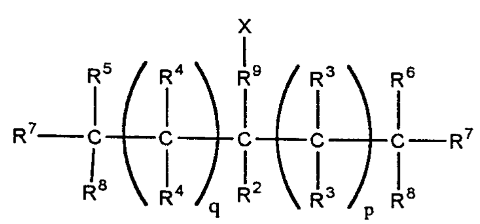
Preferably e is 2 or 3, more preferably 2.

Alternatively the ammonium phosphate estergroup VI may be replaced by a glycerol derivative of the formula VB, VC or VD defined in our earlier publication no WO-A-93/01221.

The compound of the formula I may be a small, non-polymeric compound. Such compounds may have more than one pendant group X, that is m may be more than 1, although more usually m is 1. In such compounds, although n may be more than 2, it is generally found that adequate crosslinking takes place, as for glutaraldehyde, where there are two aldehyde groups per molecule, that is n is 2. In such non-polymeric compounds, the group R^1 is generally a C_{2-12}-n+m-functional optionally substituted alkane group.

The group R^1 may be an alkyl or an aralkyl group and may be interrupted by heteroatoms such as oxygen atoms, amido and/or sulphonamido, groups, or by carbonyl groups and may be substituted by alkyl, alkoxy, aryl, aralkyl, aralkoxy, hydroxyl or alkylamido groups, or halogen atoms. Generically any substituents should have no ionic charge, under aqueous conditions at around pH 7, eg in the range 6-8 preferably 4-10. Most preferably R^1 is a C_{3-24}-alkyl group, especially a C_{3-12}-alkyl group.

Preferably the compound of the formula I is a compound of the formula VII



VII

in which X is as defined above;

R^2 is hydrogen or a C_{1-4}-alkyl group,

each of the groups R^3 and R^4 is independently selected from hydrogen, halogen, optionally substituted C_{1-24}-alkyl, and hydroxyl groups, or two groups R^3 or two groups R^4 or one group R^3 and one group R^4 together represent an optionally substituted C_{1-8}-alkylene, an optionally substituted C_{2-8}-alkenylene or an optionally substituted C_{2-8}-alkynylene group, or two groups R^3 or two groups R^4 attached to adjacent carbon atoms may, together with the carbon atoms to which they are attached form a 1,2-arylene group,

p is 1-4;

q is 1-4;

R^5 and R^6 are each independently selected from hydrogen and C_{1-4}-alkyl,

each R^7 is the same and is hydroxyl or C_{1-4}-alkoxy,

each R^8 is the same and is selected from hydroxyl and C_{1-4} alkoxy, or

the groups R^7 and R^8 attached to the same carbon atom together are =O or C_{1-4}-oxaalkyleneoxy, and

R^9 is a bond or a C_{1-24} alkylene group.

Compounds of the formula VII are thus dialdehyde compounds or the corresponding gem-diol, hemiacetal or acetal derivatives. Gem-diols, hemiacetal and acetal compounds are precursors to aldehydes in that they can react easily to form the corresponding aldehydes. These compounds are believed to be novel and form a further aspect of this invention.

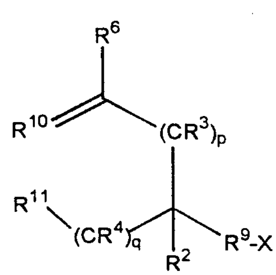
p and q are preferably each 1. Thus the group R^1 is preferably a linear C3 alkane derivative, in which the CHO groups are joined at the 1 and 3-positions whilst the zwitterionic group is joined through the 2 position.

Preferably each group R^3 and R^4 is independently selected from hydrogen, optionally substituted C_{1-24}-alkyl, hydroxyl, protected hydroxyl and amine.

Preferred substituents in alkyl groups R^3 and R^4 are hydroxyl, amino and halogen. Preferably each of R^3 and R^4 is unsubstituted C_{1-4}-alkyl or hydrogen, most preferably hydrogen.

Preferably R^5 and R^6 each represent hydrogen.

The novel compounds may be made by a process in which a compound of the formula VIII



VIII

in which a) either R^{10} is CR^{12}_2 and
R^{11} is $CR^5{=}CR^{12}_2$ or
b) R^{10} and R^{11} together are CR^5;

and in which each R^{12} is selected from hydrogen and C_{1-4} alkyl;

R^5 and R^6 are each hydrogen; and

R^3, R^4, R^9, X, q and p have the same meanings as in the compound of the formula VII,

is oxidised to form the dialdehyde compound upon oxidation at the double bond(s).

The dialdehyde compound may be reacted with a water, an alcohol or a glycol to form the corresponding gem-diol, hemiacetal or acetal, that is in which each of R^7 and R^8 represents hydroxyl or alkoxy. Such gem-diols, hemiacetals and acetals are found to be relatively stable to long term storage.

Compounds of the formula VIII believed to be novel are further claimed in our copending application filed even date herewith claiming priority from GB 97303847.4.

The oxidation reaction of the compound of the formula VIII to form the compounds of the formula VII has been found to produce stable products, where there are 3 carbon atoms between the groups $R^6C(R^7)R^8$ and $R^5C(R^7)(R^8)$ (ie q=p=1), or where there are more than 3 carbon atoms and the groups $R^6C(R^7)R^8$ and $R^5C(R^7)R^8$ are kept apart by steric means to prevent an intramolecular alcohol condensation taking place. Where q+p>2, it is preferred for one group R^3 and one group R^4 together to represent a C_{1-2}, preferably C_1-alkylene, usually methylene. The dialdehyde product of the oxidation of such compounds is resistant to an internal aldol condensation and is relatively storage stable in the form of the gem-diol, hemiacetal or acetal derivative.

The oxidation reaction may be carried out using an appropriate oxidising agent, such as ozone in conjunction with hydrogen and a palladium-carbon catalyst, with zinc and acetic acid, with iodide and acetic acid, with dimethyl sulphide, with thiourea, with triphenylphosphine, with trimethylphosphite, or with pyridine or periodate, for instance in the presence of a catalyst such as osmium tetroxide. Other oxidising agents known to generate aldehyde groups or precursors thereof from ethylenically unsaturated starting materials are potassium permanganate, sodium periodate with potassium permanganate catalyst, with ruthenium (III) chloride or ruthenium (VI) dioxide catalyst, (bi

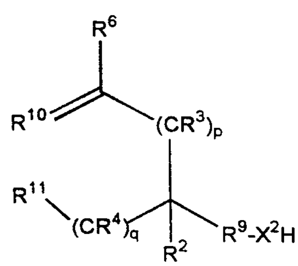
py)H_2CrOCl_5 and potassium permanganate and silica gel. These systems are further described in Comprehensive Organic Transformations, by Larock, R. C., VCH, 1989, pp595-596.

The oxidation reaction is preferably carried out with the compound of the formula VIII in solution, for instance in water or an organic solvent, or a mixture thereof. Suitable organic solvents are alcohols, dimethyl formamide or glacial acetic acid.

The oxidation reaction may be carried out with cooling. The reaction is generally carried out to completion, using an excess of oxidising agent.

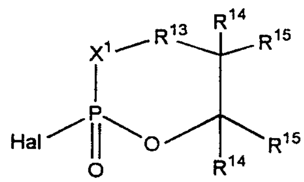
The novel starting material of the formula VIII may be made from known starting materials by various routes. In the synthetic process the zwitterionic group X is introduced into the molecule prior to oxidation of the ethylenic bond(s), using suitable chemistry. The synthesis of sulphobetaine monomer may proceed by the reaction of a precursor having a reactive group and a preformed sulphobetaine group with an ethylenically unsaturated alcohol, amine or carboxylic acid. Alternatively a sulphobetaine may be formed by reaction of a tertiary amine with a 1,3 propane sulfone.

Amino acid type compounds may be made by processes analogous to those described in our earlier application no WO-A-9416749. Where, as in the preferred aspect of the invention, the zwitterionic group is a group of formula V in which W^+ is $W^1 N^+ R^{23}_3$ which W^1 is C_2 or C_3-alkylene (optionally substituted) and at least two of the groups R^{23} are methyl, the compound of the formula VIII may be formed by the reaction of a compound of the formula IX



IX

in which the groups R^3, R^4, R^6, R^9, R^{10}, R^{11} have the same meanings as in the compound of the formula VIII, X^1 and X^2 are as defined in the group of formula V is reacted of the compound with a phospholane reagent of the formula II



X

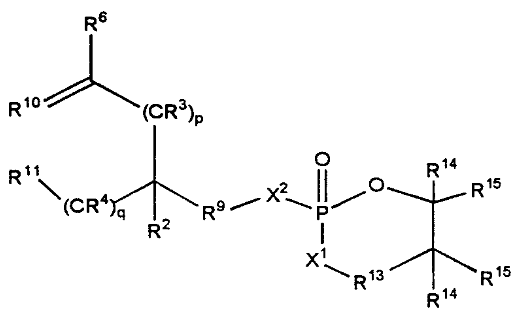
in which Hal is a halogen atom, preferably chlorine,

R^{13} is a bond or a group $C(R^{14})R^{15}$,

each group R^{14} is selected from hydrogen and C_{1-4}-alkyl groups;

each group R^{15} is selected from hydrogen and C_{1-4}-alkyl, or two groups R^{15} may form a C_{1-5}-alkylene group

to produce a phospholane intermediate of the formula X



XI

in which all the groups have the same meanings as in the respective compounds of the general formulae IX and X. Subsequently the intermediate of the formula X is subjected to a ring opening amination with an amine NR^{23}_3 reaction to produce the compound of the formula VIII.

In the phospholane reagent of the formula X, the groups R^{14} and R^{15} are preferably all the same, each preferably being hydrogen.

Where R^5 is $N^+R^6_3$, the ring opening reaction is carried out under anhydrous conditions. Preferably each of the groups R^{23} is methyl. This basic phospholane ring opening reaction has been described by Thuong and Chabrier in Bull. Soc. Chim. de France (1974) (3-4) 667-670 and in FR-A-2,270,887.

The compound of the formula VII is preferably used as a crosslinking reagent for reacting with substrates having pendant primary amine groups, especially proteinaceous substrates. In this application of the compound of the formula VII, it is preferred for the reaction to be conducted in an aqueous environment.

The invention is illustrated in the following examples:

Example 1 - <u>Synthesis of 3-Methoxy-(ethyl-2'-(trimethylammonium ethyl) phosphate) - hexan-1,6-dial</u>

1.1: <u>Synthesis of 3-Cyclohexen-1-methoxyethyl-2'-(trimethylammonium ethyl) phosphate</u>

1.1 To a solution of +/- 3-cylcohexen-1-methanol (2.60g, 0.023mole) in dry acetonitrile (60ml) and triethylamine (4.00g, 0.039mole) at -10°C, a solution of 2-chloro-2-oxo-1,3,2-dioxaphospholane (CCP) (4.37g, 0.030mole) in acetonitrile (15ml) was slowly added. The reaction mixture was allowed to warm to RT over a period of 1 h. The solids were removed by filtration, and the solution added to a flask containing trimethylamine (2.4g, 0.041mole) in acetonitrile (50ml). The resulting mixture was heated at 50°C overnight. The solvents were removed *in vacuo* to afford a viscous oil (7.10g, 0.025mole), contaminated by slight amounts of trimethylamine.hydrochloride, and acetonitrile:

^{1}H NMR (199.5MHz, $CDCl_3$), 1.2 (1H, m, CH), 1.6-2.2 (6H, m, 3xCH_2), 3.43 (9H, s, NMe_3), 3.74 (2H, m, CH_2OP), 3.8-4.6 (4H, m, OCH_2-CH_2-N), 5.65 (2H, s, CH=CH).

^{13}C NMR (50.1MHz, D_2O), 23($CHCH_2CH_2CH$=), 25($CHCH_2CH_2CH$=), 27(=$CHCH_2CH$), 33(CH), 53(NMe_3), 59($POCH_2$), 65(CH_2N), 70(CH_2O), 125(=$CHCH_2CH$), 127($CHCH_2CH_2CH$=).

An analogous process can be carried out using in place of the 3-cyclohexene-1-methanol, 3-methyl-2-cyclohexen-1 methanol or 2-cyclohexen-1-ol or 3,-methyl 5,5 tri-2-cyclohexen-1-methanol.

1.2: Synthesis of 3-Methoxy-(ethyl-2'-(trimethylammonium ethyl) phosphate) -hexan-1,6-dial

1.2.1 - Osmium tetroxide.

To a solution of 3-cyclohexen-1-methoxyethyl-2'-(trimethylammonium ethyl) phosphate (2.771g, 0.0099M) in 1,4-dioxane / water (50ml, 1:1, v/v), a grain of osmium tetroxide (<1mg) was added, and the solution stirred for 30 min. Sodium periodate (6.727g, 0.030mole) was slowly added over 30 min and the reaction stirred for 6 h. The solid material was filtered off, and the solvent removed *in vaccuo* to give a white solid. The solid was dissolved in ethanol (50ml) and again filtered. The solution was concentrated to give a viscous oil (3.03g, 0.0097mole) still containing some solvent and sodium iodide salts:

^{1}H NMR (199.5MHz, D_2O), 1.3-1.9 (7H, m, $CH_2CH_2CHCH_2$), 3.23 (9H, s, NMe_3), 3.7-4.3 (6H, $CH_2OPOCH_2CH_2N$), 9.7(2H, CH=O).

^{13}C NMR (50.1MHz, D_2O) 30.8, 45.2, 53.4(ref NMe_3), 59.0, 60.3, 65.6, 66.7, 206.1. IR (KBr) 3401, 2946, 1716, 1670, 1479, 1398, 1220, 1040, 971, 875, 765, 666, 502.

1.2.2 - Ozone.

Ozone was bubbled through a solution 3-cyclohexen-1-methoxyethyl-2'-(trimethylammonium ethyl) phosphate (7.52g, 0.027mole) in ethanol (200ml) at -78°C, for 5 h. Triphenylphosphine (14.0g, 0.053mole) was added to quench the reaction mixture, whereupon the reaction darkened from colourless to yellow, and then a fine white precipitate was observed. The reaction mixture was stirred for 48 h. The reaction mixture was filtered, and the solvents removed *in vacuo*. Dissolution of the resulting oil proved problematic, and ^{1}H NMR analysis of the reaction mixture showed a complex mixture of signals indicating that there may be decomposition of the starting material.

Example 2

Synthesis of 3-(Oxyethyl-2'-(trimethylammonium ethyl) phosphate)-pentan-1, 5-dial

2.1-2.2: Synthesis of 3-Cyclopenten-1-ol

2.1 To a stirred solution of cyclopentadiene (57.8g, 0.87mole), and sodium carbonate (400g) in dichloromethane (1000ml) at 0°C, peracetic acid (168ml, 40% in acetic acid), pre-treated with sodium acetate (0.2g) was added slowly, and the reaction allowed to stir for 4 h. The reaction mixture was filtered, and the solvent distilled off.

[^{1}H NMR (199.5MHz, $CDCl_3$) 2.0-3.0(m, C*H*-O-C*H*), 3.8(d, CH_2), 6.1(m, C*H*=C*H*)]

2.2 The residue was slowly added to a slurry of lithium aluminium hydride (13.0g, 0.36mole) in diethyl ether (400ml) at 0°C, and stirred overnight. The reaction was quenched by slow addition of water (50ml), and then after 10 min dried magnesium sulphate was added (50-70g). The solids were filtered off, and then washed with diethyl ether (2x200ml). The organic layers were combined, and the solvent was removed carefully *in vacuo* (no heating). 3-Cyclopenten-1-ol (20.38g, 0.243mole) was isolated from the mixture by distillation (27°C, *ca* 754 mmHg).

^{1}H NMR (199.5MHz, $CDCl_3$) 1.9(1H, br.s, O*H*), 2.2-2.8(4H, qd, CH_2CHCH_2), 4.5(1H, br.m, C*H*OH), 5.7(2H, s, C*H*=C*H*).

^{13}C NMR (50.1MHz, $CDCl_3$) 42.0 (*C*H_2), 10.8 (*C*HOH), 122.4(*C*=*C*).

2.3: Synthesis of 3-Cyclopenten-1-oxyethyl-2'-(trimethylammonium ethyl) phosphate

To a solution of 3-cyclopenten-1-ol (8.04g, 0.091mole), and *N,N,N,N*-tetramethylethylenediamine (6.33g, 0.055mole) in acetonitrile (160ml) at -10°C, a solution of 2-chloro-2-oxo-1,3,2-dioxaphospholane (15.6g, 0.073mole) in acetonitrile (50ml) was added, and the reaction allowed to warm to RT over 1.5 h. The reaction mixture was filtered, and then trimethylamine (12.68g, 0.21mole) was added. The reaction was heated at 50°C in a closed system for 18 h. The reaction was cooled, and solvents removed *in vacuo*. The reaction mixture was taken up into water, and washed

with chloroform (2x100ml). The aqueous layer was concentrated to yield 3-cyclopenten-1-oxyethyl-2'-(trimethylammonium ethyl) phosphate (15.3g, 0.061mole) as an oil.

^1H NMR (199.5MHz,D_2O) 2.1-2.4(4H, m, 2x-CH_2-), 3.0(9H, s, NMe_3), 3.3-4.1(5H, m, *C*H*O*P, OCH_2CH_2N), 5.5(2H, s, *CH*=*CH*).

^{13}C NMR (50.1MHz, D_2O) 39.5(CH_2), 53(ref NMe_3), 58.5&60.0, 65.0&66.5(OCH_2CH_2N), 75.5(*C*HOP), 127.2(*C*=*C*).

m/s (FAB^+) 336(M^+•NMe_3•H_2O), 250(M^+), 185

Ir (KBr smear) 3401, 2959, 2510, 1653, 1483, 1220, 991, 769, 668, 510.

2.4: Synthesis of 3-(Oxyethyl-2'-(trimethylammonium ethyl) phosphate)-pentan-1, 5-dial (ethoxyacetalderivative)

To a solution of 3-cyclopenten-1-oxyethyl-2'-(trimethylammonium ethyl) phosphate (2.43g, 0.0097mole) in water (100ml), a grain of osmium tetroxide (<1mg) was added, and the reaction stirred at RT for 30 min. Sodium periodate (5.43g, 0.0254mole) was added slowly, and the reaction stirred at RT for 3 h, during which a colour change to dark orange, and then back to colourless was observed. Ethanol (200ml) was added to quench the reaction, and the precipitate was filtered off. The solvent was removed *in vacuo* to afford quantitative yield of 1,1,5,5-tetraethoxy-3-(oxyethyl-2'-(trimethylammonium ethyl) phosphate)-pentane (based on ^{13}C NMR), i.e. the ethoxy acetal derivative of the dialdehyde.

^1H NMR (199.5MHz,D_2O) 1.1-2.0(4H, m, 2xCH_2), 3.0(9H, s, NMe_3), 3.3-4.2(7H, m, C*H*OP, OCH_2CH_2N, C*H*(O(D))$_2$).

^{13}C NMR (50.1MHz, D_2O) 17(CH_3CH_2O), 35&37(-CH_2-), 53(refNMe_3), 57(CH_3CH_2O), 58&60(CH_2OP), 65&66(CH_2N), 70(*C*HOP), 90(*C*H(OEt)$_2$).

IR (KBr smear) 3401, 2505, 2360, 1653, 1481, 1218, 1085, 971, 759, 513.

Example 3 - Tissue interaction

3.1 Tissue Preparation:

The tissue was stored in phosphate buffer solution (PBS) for 3 hours and then washed in fresh PBS prior to cutting. The tissue was cut into strips of 1 cm by 6 cm. Eighteen strips was used for the fixation solutions and the rest stored in PBS, in the fridge, as control samples. A small sample of tissue from each of the three solutions was investigated by Differential Scanning Calorimetry (DSC).

3.2 Preparation of Fixation solutions:

The fixation solutions of 0.35 %w/v glutaraldehyde and 1.03 %w/v 3-[(oxy)(1,1,1 trimethyl ammonium) phosphoranate]- pentan-1, 5-dial (Glut-PC) in PBS was prepared as follows -

A. Glutaraldehyde solution contains 0.35% w/v glutaraldehyde and 154mM sodium chloride and has a pH in the range 7.0 to 7.5.

B. Glut-PC solution contains 1.03% w/v Glut PC (equimolar with glutaraldehyde), 154mM sodium chloride and 5.26mM trisodium phosphate for adjusting the pH to be in the range 6.5 to 7.0.

Two sets of 9 strips of tissue were placed into vials containing the control fixation solution (45mls) or the test solution. Each vial was then stored inside a brown-glass bottle for 96 hours at 25 °C.

3.3 Method for testing the Cross-linked tissue:

A modified method of testing the shrinkage temperature of leather (BSI 3144:1968 Sampling and Physical Testing of Leather) was used to test the tissue.

The three types of tissues were suspended in a beaker containing a solution of PBS with a thermometer placed at close proximity to the tissue. The solution of PBS was heated in water at a constant power to provide a temperature rise of approximately 2 °C/min from 40 °C to 90 °C. The change in physical appearances of the samples was recorded prior to testing and recorded in Table 1. Also a thermometer positioned close to the sample was used to measure the temperature rise with time. Inflexion points in the curve were recorded and are reported in Tables 2-4. The results of the DSC test is reported in Table 5.

Results

Physical Appearance prior to testing:

Table 1

Tissue Type	Physical appearance
Natural tissue	Pink/white colour. Soft, flexible texture to the tissue.
Glutaraldehyde fixed	Brown colour and stiff/firm texture of tissue.
Glut-PC fixed	Dark red/orange colour with a slightly firmer texture compared to natural tissue. The tissue is different in colour; and more softer and flexible compared to the glutaraldehyde fixed tissue.

Experimental Results:

Experiment 1

Table 2

Starting temperature - 40°C Final Temperature - 89°C

Temperature/ °C	Sample
60 ± 2°C	Natural Tissue - single transition
56 ± 1°C 60 ± 1°C 65 ± 2°C	Glut-PC preserved - three transitions observed.
80 ± 2°C	Glutaraldehyde preserved - single transition

Experiment 2

Table 3

Starting temperature - 36.5°C Final temperature - 88.5°C

Temperature	Sample
58 ±± 1°C	Natural Tissue - single transition
53 ±± 1°C 61 ±± 1°C 68 ±± 1°C	Glut-PC preserved - three transitions observed.
80 ±± 2°C	Glutaraldehyde preserved - single transition

Experiment 3:

Table 4

Starting temperature - 36°C Final Temperature - 86°C

Temperature/ °C	Sample
60 ±± 2°C	Natural Tissue - single transition
56 ±± 1°C 59 ±± 1°C 63 ±± 1°C 68 ±± 1°C	Glut-PC preserved - four transitions observed
84 ±± 2°C	Glutaraldehyde preserved - single transition.

DSC Experimentation-

Conditions – Sealed pans containing samples.

Heating 25°C - 100°C at 10°C/minute.

Table 5

Temperature/ °C	Sample
~75	Natural Tissue – single transition
~65	Glut-PC preserved – single transition
~85	Glutaraldehyde preserved - single transition.

The results show that the PC group containing compound performs differently to glutaraldehyde. The physical properties of the treated tissue appear to be more similar to those of the untreated tissue, which is believed to be desirable.

Example 4

4.1 Synthesis of 5-[(oxy)-(1,1,1 trimethyl ammonium) phosphoranate methyl]-bicyclo [2.2.1] hept-2-ene.

To a solution of 5-norbornene-2-methanol (1.00 g, 0.0079 mol) and N,N,N',N'-tetramethylethylenediamine (0.46 g, 0.0038 mol) in acetonitrile (30 ml) at 0 °C, a solution of 2-chloro-1,3,2-dioxaphospholane-2-oxide (1.13 g, 0.0079 mol) in MeCN (10 ml) was slowly added over 20 minutes under N_2. The solution was stirred for 4h. The solution was filtered and cooled to 0 °C. Trimethylamine (1.17 g, 0.0198 mol) was added and the solution heated at 50 °C overnight in a closed system. The solution was cooled, degassed, and the solution decanted. The solvent was evaporated and the residue partitioned between H_2O (20 ml) and Et_2O (20 ml). The aqueous layer was washed with Et_2O (20 ml), separated and evaporated. The yellow oil was identified as the product:
^1H NMR (400 MHz, D_2O) δ complex spectra.
^{13}C NMR (50.1 MHz, D_2O) δ 27.0 ($\underline{C}H_2CH(CH_2O)$), 38.0 ($\underline{C}HCH_2OP$), 41.8 ($\underline{C}(CH_2)CH$), 43.5 ($\underline{C}(CH_2)CH_2$), 47.8 ($CH_2$ bridge), 54.0 ($\underline{Me_3}N$), 58.1 ($\underline{C}H_2NMe_3$), 64.8 ($\underline{C}H_2OP$), 68.5 ($CH\underline{C}H_2OP$), 131.1 ($\underline{C}HCH$, *trans*), 135.4 ($\underline{C}HCH$, *cis*), 136.2 ($CH\underline{C}H$, *cis*) 136.7 ($CH\underline{C}H$, *trans*).

4.2 Synthesis of 4-[(oxy)(1,1,1 trimethyl ammonium) phosphoranate methyl]-cyclopentan-1, 3-dicarbaldehyde.

A 5% solution of 5-[(oxy)-(1,1,1 trimethyl ammonium) phosphoranate methyl]-bicyclo

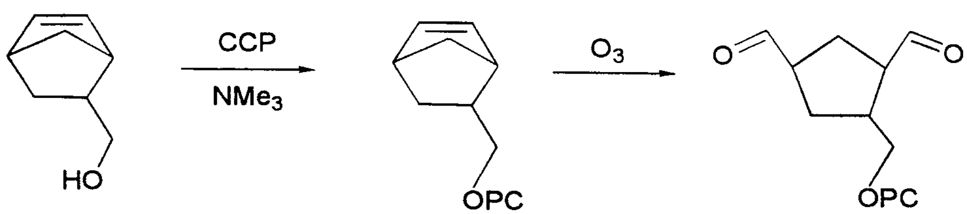
[2.2.1] hept-2-ene (1.00 g, 0.0035 mol) in H_2O (20 ml) was subjected to a stream of O_3 (0.0017 mol/min, 2 min.) at 5 °C. Triphenyl phosphine (1.00 g, 0.0038 mol) was added and stirred for 1h at room temperature. The solution was filtered, frozen and the water removed by freeze-drying.

^{1}H NMR (400 MHz, D_2O) δ complex spectra.

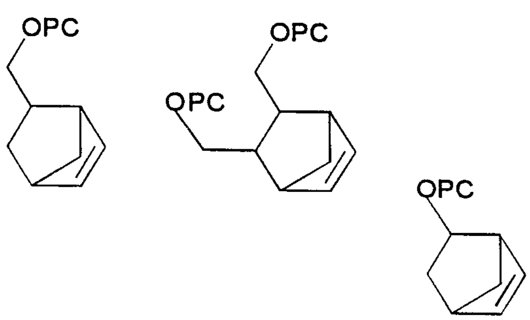
^{13}C NMR (50.1 MHz, D_2O) δ 29.3 (<u>C</u>H_2CH(CH_2O)), 41.2 (<u>C</u>HCH_2OP), 43.5 (<u>C</u>(CH_2)CH), 44.7 (<u>C</u>(CH_2)CH_2), 51.1 (CH_2 bridge), 54.0 (<u>Me</u>$_3$N), 59.5 (<u>C</u>H_2NMe_3), 61.2 (<u>C</u>H_2OP), 66.0 (CH<u>C</u>H_2OP), 97.4 & 101.4 (<u>C</u>H$(OH)_2$).

DNPH test - yellow precipitate formed (positive result).

Proton and carbon nmr are consistent with the following proposed reaction scheme



Analogous methods to those of Example 4.1 may be used to make the following cycloalkene compounds



From these dialdehydes may be formed using techniques analogous to Example 4.2.

Example 5

5.1 Synthesis of 1,6-heptadien-4-ol:

To a suspension of activated magnesium ribbon [activated overnight by stirring in an inert atmosphere] (19.6 g, 0.82 mol) and anhydrous diethyl ether [Et_2O] (307 ml), an initiating solution of allyl bromide (10%, 9.0 g, 0.074 mol) in Et_2O (13 ml) was added. Once the reaction had started to reflux gently, the remainder of the allyl bromide (81.0 g, 0.646mol) in Et_2O(119 ml) was slowly added maintaining a gentle reflux. The reaction was left refluxing for 2h. The mixture was cooled in an ice bath and a solution of ethyl formate (24.77 g, 0.33 mol) in Et_2O (53 ml) was slowly added to the stirred mixture over 1h. The reaction was left to warm to room temperature overnight. The reaction was quenched with saturated ammonium chloride solution (260 ml), and H_2O then added (250 ml). The aqueous layer was acidified with dilute (0.1 M) hydrochloric acid and the organic layer separated. The organic layer was washed with $NaHCO_3$ (300 ml), H_2O (200 ml), and NaCl (200 ml). The organic layer was dried with anhydrous sodium sulphate and the solvent removed. The resulting pale yellow oil was identified as a mixture of the alcohol and the formate ester (approximately 5:2 ratio):

^1H NMR (400 MHz, $CDCl_3$) δ 1.90 (1H, m, CHO$\underline{H}$), 2.20 (2H,m, CHC$\underline{H}_2$), 2.35 (2H, m, CHC$\underline{H}_2$), 3.70 (1H, m, C$\underline{H}$OH), 5.15 (4H, m, 2xCHC$\underline{H}_2$), 5.75 (2H, m, 2xC$\underline{H}CH_2$), 8.05 (1H, s, OC($\underline{H}$)-O-CH).

^{13}C NMR (50.1 MHz, $CDCl_3$) δ 37.8 ($\underline{C}H_2$), 41.2 ($\underline{C}H_2$), 69.7 ($\underline{C}$HOH), 72.4 ($\underline{C}$HO-COH), 118.1 ($\underline{C}HCH_2$), 118.3 ($\underline{C}HCH_2$), 133.0 (CH$\underline{C}H_2$), 134.6 (CH$\underline{C}H_2$), 160.7 (OC($\underline{H}$)-O-CH).

5.2 Synthesis of 4-[(oxy)(1,1,1 trimethyl ammonium) phosphoranate]-1,6-heptadiene.

To a solution of 1,6-heptadien-4-ol (10.00 g, 0.089 mol) and N,N,N',N'-tetramethylethylenediamine (5.18 g, 0.045 mol) in acetonitrile [MeCN] (100 ml) at 0 °C, a solution of 2-chloro-1,3,2-dioxaphospholane-2-oxide (12.72 g, 0.089 mol) in MeCN (60 ml) was slowly added over 20 minutes under N_2. The solution was stirred for 4h. The solution was filtered and cooled to 0 °C. Trimethylamine (13.17 g, 0.223 mol) was added and the solution heated at 50 °C overnight in a closed system. The solution was cooled, degassed, and the solution decanted. The solvent was evaporated and the residue partitioned between H_2O (150 ml) and dichloromethane [DCM] (150 ml). The aqueous

layer was washed with DCM (150 ml), separated and evaporated. The residue was washed with acetone (2x 150 ml) and stored in acetone (100 ml) overnight. The acetone layer was evaporated to leave a solid residue. The solid residue was identified as the product:

^{1}H NMR (400 MHz, D_2O) δ complex spectra.

^{13}C NMR (50.1 MHz, D_2O) δ 43.5 (<u>C</u>H_2), 54.0 (<u>Me$_3$</u>NCH_2), 55.5 (<u>C</u>H_2NMe_3), 59.8 (<u>C</u>H_2OPO), 65.7 (<u>C</u>HOP), 118.1 (<u>C</u>HCH_2), 134.2 (CH<u>C</u>H_2).

5.3 <u>Synthesis of 3-[(oxy)(1,1,1 trimethyl ammonium) phosphoranate]- pentan-1,5-dial.</u>

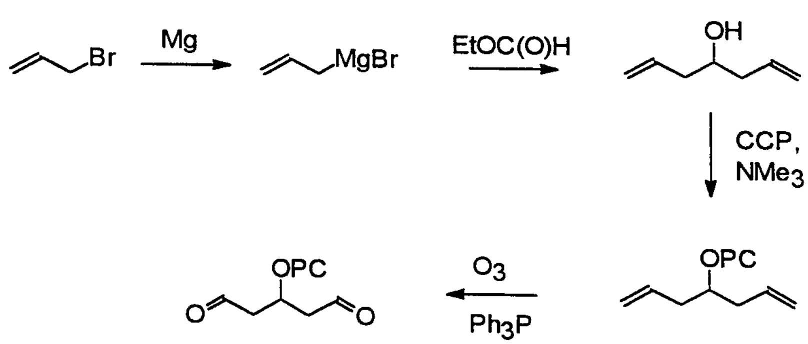
A 10% solution of 4-[(oxy) (1,1,1 trimethyl ammonium) phosphoranate]-1,6-heptadiene (2.00 g, 0.0073 mol) in H_2O (20 ml) was subjected to a stream of O_3 (0.0017 mol/min, 10 min.) at 5 °C. Triphenyl phosphine (4.75 g, 0.018 mol) was added and stirred for 1h at room temperature. The solution was filtered, frozen and the water and formaldehyde removed by freeze-drying.

^{1}H NMR (400 MHz, D_2O) δ complex spectra.

^{13}C NMR (50.1 MHz, D_2O) δ 43.8 (<u>C</u>H_2), 54.0 (<u>Me$_3$</u>NCH_2), 55.6 (<u>C</u>H_2NMe_3), 60.0 (<u>C</u>H_2OPO), 65.8 (<u>C</u>HOP), 91.8 & 92.7 (<u>C</u>H$(OH)_2$).

DNPH test - orange precipitate formed. [Formaldehyde produces a bright yellow precipitate]

The reaction is believed to take place according to the following scheme:



Example 6

6.1 Synthesis of 3, 5-dimethyl-1,6-heptadien-4-ol:

To a suspension of activated magnesium ribbon [activated overnight by stirring in an inert atmosphere] (6.22 g, 0.26 mol) and anhydrous tetrahydrofuran [THF] (110 ml), an initiating solution of 3 chloro-1-butene (10%, 2.0 g, 0.02 mol) in THF (6 ml) and a crystal of iodine were added. Once the reaction had started to reflux gently, the remainder of the 3 chloro-1-butene (18.0 g, 0.199 mol) in THF (51 ml) was slowly added maintaining a gentle reflux. The reaction was left refluxing for 2h and heated further at 70 °C for 1 h. The mixture was cooled to room temperature and a solution of ethyl formate (7.38 g, 0.10 mol) in THF (20 ml) was slowly added to the stirred mixture over 1h. The reaction was left overnight. The reaction was quenched with saturated ammonium chloride solution (270 ml). The organic layer was separated and washed with NaHCO3 (275 ml) and brine solution (250 ml). Ethyl acetate (300 ml) was added and organic layer separated. The organic layer was treated with charcoal, filtered with celite and the solvent removed. The resulting yellow oil was believed to be a mixture of the three types of alcohol and their ester derivatives:

^{1}H NMR (400 MHz, $CDCl_3$) complex spectra. δ 0.9 - 1.10 (3H, m, CH<u>Me</u>), 1.60 (3H, d, <u>Me</u>CH=), 2.20 (2H,m, CHC<u>H</u>$_2$), 2.35 - 2.55 (2H, m, CHC<u>H</u>$_2$), 3.25 - 3.70 (1H, m, C<u>H</u>OH), 5.00 - 5.15 (4H, m, 2xCHC<u>H</u>$_2$), 5.65 - 5.85 (2H, m, 2xC<u>H</u>CH$_2$ and MeCHCH), 8.05 - 8.15 (1H, s, OC(<u>H</u>)-O-CH).

^{13}C NMR (50.1 MHz, $CDCl_3$) complex spectra. δ 15 - 20 (<u>C</u>H$_3$), 38.0 - 41.5 (<u>C</u>H$_2$ and <u>C</u>HMe), 75 - 80 (<u>C</u>HOH and <u>C</u>HO-COH), 114 - 118 (<u>C</u>HCH$_2$ and <u>C</u>HCHMe), 138 - 141 (CH<u>C</u>H$_2$ and CH<u>C</u>HMe), 160.7 (OC(<u>H</u>)-O-CH).

6.2 Synthesis of 4-[(oxy)-(1,1,1 trimethyl ammonium) phosphoranate]-3, 5-dimethyl-1,6-heptadiene.

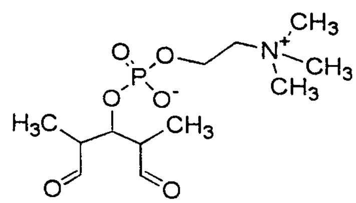
To a solution of 3, 5-dimethyl-1,6-heptadien-4-ol (2.00 g, 0.014 mol) and N,N,N',N'-tetramethylethylenediamine (0.83 g, 0.007 mol) in acetonitrile [MeCN] (20 ml) at 0 °C, a solution of 2-chloro-1,3,2-dioxaphospholane-2-oxide (2.04 g, 0.14 mol) in MeCN (25 ml) was slowly added over 20 minutes under N_2. The solution was stirred for 4h. The solution was filtered and cooled to 0 °C. Trimethylamine (3.10 g, 0.053 mol) was added and the solution heated at 50 °C overnight in a closed system. The solution was cooled, degassed and the solution decanted. The solvent was evaporated and the

residue partitioned between H_2O (125 ml) and ether [Et_2O] (130 ml). The aqueous layer washed with Et_2O (130 ml), separated and evaporated. The residue was identified as a phosphorous species, but without the dialkene.

^1H NMR (400 MHz, D_2O) δ complex spectra.

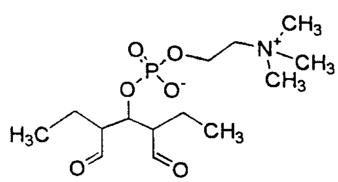
^{13}C NMR (50.1 MHz, D_2O) δ 54.0 ($\underline{Me_3}NCH_2$), 55.0 ($\underline{C}H_2NMe_3$), 62.2 ($\underline{C}H_2OPO$).

The product contained several isomers and was not further treated. The product could be separated into the individual isomers and be subjected to ozonolysis to form analogous dialdehydes to those of example 5, for instance having the formula

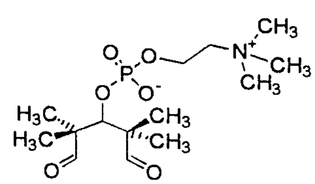


A

Analogous methods may be used to make the following PC dialdehyde compounds



B



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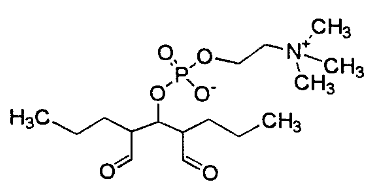
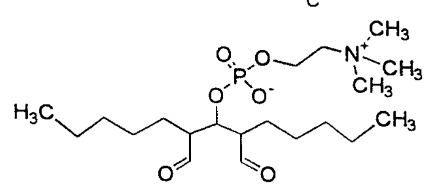


D



E

CLAIMS

1. A process in which a zwitterionic crosslinker of the formula I

$$X_m (R^1) (CHO)_n \qquad I$$

in which X is a zwitterionic pendant group having an overall neutral charge,

R^1 is an organic group having n+m functionality,

m is at least 1 and

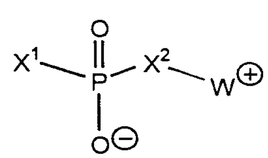
n is at least 2,

or a gem-diol, hemiacetal or acetal derivative thereof, is contacted in aqueous solution with a substrate having pendant primary amine groups, under conditions allowing reaction of the CHO groups of the compound of the formula I with the primary amine groups of the substrate.

2. A process according to claim 1 in which the substrate comprises protein, preferably comprising collagen.

3. A process according to claim 1 or claim 2 in which the zwitterionic group comprises a phosphate group as the anion and a quaternary ammonium group at the cation.

4. A process according to claim 1 or claim 2 in which X has the general formula V



V

in which the moieties X^1 and X^2, which are the same or different, are -O-, -S-, -NH- or a valence bond, preferably -O-, and W^+ is a group comprising an ammonium, phosphonium or sulphonium cationic group having a group linking the anionic and cationic moieties which is a C_{1-12} alkylene group optionally containing one or more ethylenically unsaturated double or triple bonds, disubstituted-aryl, alkylene aryl, aryl alkylene, or alkylene aryl alkylene, disubstituted cycloalkyl, alkylene cycloalkyl, cycloalkyl alkylene or alkylene cycloalkyl alkylene, optionally containing one or more fluorine substituents.

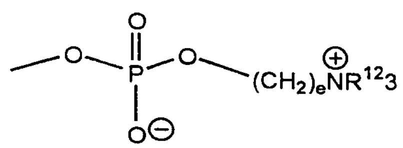
5. A process according to claim 4 in which W^+ is a group of formula $-W^1-N^+R^{23}_3$, $-W^1-P^+R^{23a}_3$, $-W^1-S^+R^{23a}_2$ or $-W^1-Het^+$ in which:

either the groups R^{23} are the same or different and each is hydrogen or alkyl of 1 to 4 carbon atoms or aryl, or two of the groups R^{23} together with the heteroatom to which they are attached form a heterocyclic ring containing from 5 to 7 atoms or the three groups R^{23} together with the heteroatom to which they are attached form a fused ring structure containing from 5 to 7 atoms in each ring, and optionally one or more of the groups R^{23} is substituted by a hydrophilic functional group, and

the groups R^{23a} are the same or different and each is R^{23} or a group OR^{23}, where R^{23} is as defined above; or

Het is an aromatic nitrogen-, phosphorus- or sulphur-, preferably nitrogen-, containing ring.

6. A process according to claim 5 in which X is a group of formula VI:



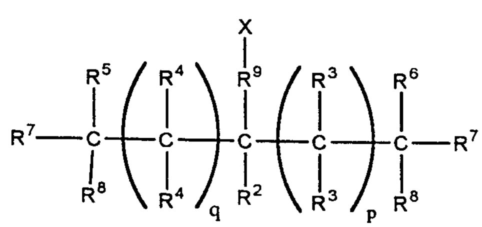
VI

where the groups R^{12} are the same or different and each is hydrogen or C_{1-4} alkyl, and e is from 1 to 4, preferably in which e is 2 or 3 and each R^{12} is methyl.

7. A process according to any preceding claim in which m is 1.

8. A process according to any preceding claim in which R^1 is a C_{3-24}, preferably a C_{3-12}-alkyl group.

9. A process according to any preceding claim in which the compound has the general formula VII



VII

in which X is as defined in any of claims 1 and 3 to 6

R^2 is hydrogen or a C_{1-4}-alkyl group,

each of the groups R^3 and R^4 is independently selected from hydrogen, halogen, optionally substituted C_{1-24}-alkyl, and hydroxyl groups, or two groups R^3 or two groups R^4 or one group R^3 and one group R^4 together represent an optionally substituted C_{1-8}-alkylene, an optionally substituted C_{2-8}-alkenylene or an optionally substituted C_{2-8}-alkynylene group, or two groups R^3 or two groups R^4 attached to adjacent carbon atoms may, together with the carbon atoms to which they are attached form a 1,2-arylene group,

p is 1-4;

q is 1-4;

R^5 and R^6 are each independently selected from hydrogen and C_{1-4}-alkyl,

each R^7 is the same and is hydroxyl or C_{1-4}-alkoxy,

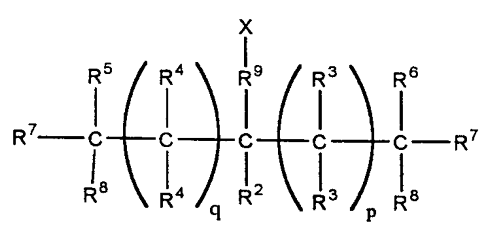
each R^8 is the same and is selected from hydroxyl and C_{1-4} alkoxy, or

the groups R^7 and R^8 attached to the same carbon atom together are =O or C_{1-4}-oxaalkyleneoxy, and

R^9 is a bond or a C_{1-24} alkylene group.

10. A process according to claim 9 in which m and n are both 1, R^3 and R^4 are selected from hydrogen and C_{1-4} alkyl and R^5 and R^6 are each hydrogen.

11. A compound of the general formula VII



VII

X is a zwitterionic group having an overall neutral charge;

R^2 is hydrogen or a C_{1-4}-alkyl group,

each of the groups R^3 and R^4 is independently selected from hydrogen, halogen, optionally substituted C_{1-24}-alkyl, and hydroxyl groups, or two groups R^3 or two groups R^4 or one group R^3 and one group R^4 together represent an optionally substituted C_{1-8}-alkylene, an optionally substituted C_{2-8}-alkenylene or an optionally substituted C_{2-8}-alkynylene group, or two groups R^3 or two groups R^4 attached to adjacent carbon

atoms may, together with the carbon atoms to which they are attached form a 1,2-arylene group,

p is 1-4;

q is 1-4;

R^5 and R^6 are each independently selected from hydrogen and C_{1-4}-alkyl,

each R^7 is the same and is hydroxyl or C_{1-4}-alkoxy,

each R^8 is the same and is selected from hydroxyl and C_{1-4} alkoxy, or

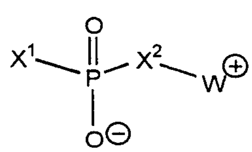
the groups R^7 and R^8 attached to the same carbon atom together are =O or C_{1-4}-oxaalkyleneoxy, and

R^9 is a bond or a C_{1-24} alkylene group.

12. A compound according to claim 11 in which p and q are both 1, R^3 and R^4 are selected from hydrogen and C_{1-4} alkyl and R^5 and R^6 are each hydrogen.

13. A compound according to claim 11 or claim 12 in which the zwitterionic group comprises a phosphate group as the anion and a quaternary ammonium group as the cation.

14. A compound according to claim 11 or claim 12 in which X has the general formula V



V

in which the moieties X^1 and X^2, which are the same or different, are -O-, -S-, -NH- or a valence bond, preferably -O-, and W^+ is a group comprising an ammonium, phosphonium or sulphonium cationic group having a group linking the anionic and cationic moieties which is a C_{1-12} alkylene group optionally containing one or more ethylenically unsaturated double or triple bonds, disubstituted-aryl, alkylene aryl, aryl alkylene, or alkylene aryl alkylene, disubstituted cycloalkyl, alkylene cycloalkyl, cycloalkyl alkylene or alkylene cycloalkyl alkylene, optionally containing one or more fluorine substituents.

15. A compound according to claim 14 in which W^+ is

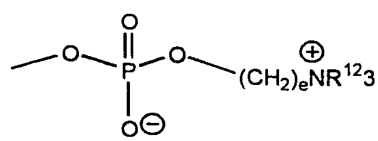
a group of formula $-W^1-N^+R^{23}_3$, $-W^1-P^+R^{23a}_3$, $-W^1-S^+R^{23a}_2$ or $-W^1-Het^+$ in which W^1 is C_{2-6}-alkylene and

either the groups R^{23} are the same or different and each is hydrogen or alkyl of 1 to 4 carbon atoms, or aryl, or two of the groups R^{23} together with the nitrogen atom to which they are attached form a heterocyclic ring containing from 5 to 7 atoms or the three groups R^{23} together with the nitrogen atom to which they are attached form a fused ring structure containing from 5 to 7 atoms in each ring, and optionally one or more of the groups R^{23} is substituted by a hydrophilic functional group, and

the groups R^{23a} are the same or different and each is R^{23} or a group OR^{23}, where R^{23} is as defined above; or

Het is an aromatic nitrogen-, phosphorus- or sulphur-, preferably nitrogen-, containing ring.

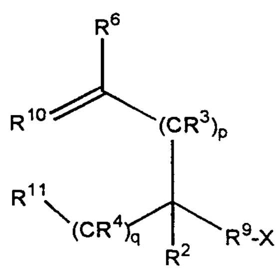
16. A compound according to claim 15 in which X is a group of formula VI:



VI

where the groups R^{12} are the same or different and each is hydrogen or C_{1-4} alkyl, and e is from 1 to 4, preferably in which e is 2 or 3 and each R^{12} is methyl.

17. A process in which a compound of the formula VIII



VIII

in which a) either R^{10} is CR^{12}_2 and R^{11} is $CR^5=CR^{12}_2$ or

b) R^{10} and R^{11} together are CR^5;

and in which each R^{12} is selected from hydrogen and C_{1-4} alkyl;

X is a zwitterionic group having an overall neutral charge;

each of the groups R^3 and R^4 is independently selected from hydrogen, halogen, optionally substituted C_{1-24}-alkyl, and hydroxyl groups, or two groups R^3 or two groups R^4 or one group R^3 and one group R^4 together represent an optionally substituted C_{1-8}-alkylene, an optionally substituted C_{2-8}-alkenylene or an optionally substituted C_{2-8}-alkynylene group, or two groups R^3 or two groups R^4 attached to adjacent carbon atoms may, together with the carbon atoms to which they are attached form a 1,2-arylene group,

p is 1-4;

q is 1-4;

R^9 is a bond or a C_{1-24} alkylene group.

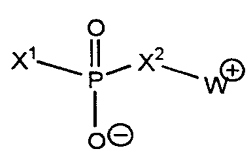
18. A process according to claim 17 in which R^{10} is CR^{12}_2 and R^{11} is $CR^5{=}CR^{12}_2$ and each R^{12} is hydrogen.

19. A process according to claim 17 in which R^{10} and R^{11} together are CR^5.

20. A process according to any of claims 17 to 19 in which p and q are both 1, and R^3 and R^4 are selected from hydrogen and C_{1-4} alkyl.

21. A process according to any of claims 17 to 20 in which the zwitterionic group comprises a phosphate group as the cation and a quaternary ammonium group as the cation.

22. A process according to any of claims 17 to 20 in which X has the general formula V



V

in which the moieties X^1 and X^2, which are the same or different, are -O-, -S-, -NH- or a valence bond, preferably -O-, and W^+ is a group comprising an ammonium, phosphonium or sulphonium cationic group having a group linking the anionic and cationic moieties which is a C_{1-12} alkylene group optionally containing one or more ethylenically unsaturated double or triple bonds, disubstituted-aryl, alkylene aryl, aryl

alkylene, or alkylene aryl alkylene, disubstituted cycloalkyl, alkylene cycloalkyl, cycloalkyl alkylene or alkylene cycloalkyl alkylene, optionally containing one or more fluorine substituents.

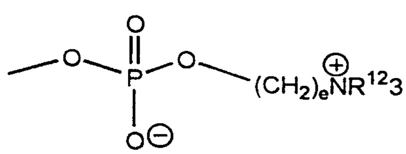
23. A process according to claim 22 in which W^+ is a group of formula $-W^1-N^+R^{23}_3$, $-W^1-P^+R^{23a}_3$, $-W^1-S^+R^{23a}_2$ or $-W^1-Het^+$ in which W^1 is C_{2-6}-alkylene and

either the groups R^{23} are the same or different and each is hydrogen or alkyl of 1 to 4 carbon atoms, or aryl, or two of the groups R^{23} together with the nitrogen atom to which they are attached form a heterocyclic ring containing from 5 to 7 atoms or the three groups R^{23} together with the nitrogen atom to which they are attached form a fused ring structure containing from 5 to 7 atoms in each ring, and optionally one or more of the groups R^{23} is substituted by a hydrophilic functional group, and

the groups R^{23a} are the same or different and each is R^{23} or a group OR^{23}, where R^{23} is as defined above; or

Het is an aromatic nitrogen-, phosphorus- or sulphur-, preferably nitrogen-, containing ring.

24. A process according to claim 23 in which X is a group of formula VI:



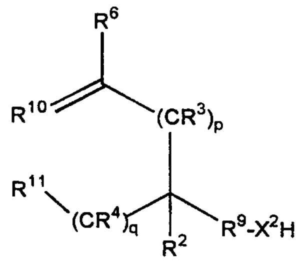
VI

where the groups R^{12} are the same or different and each is hydrogen or C_{1-4} alkyl, and e is from 1 to 4, preferably in which e is 2 or 3, and each R^{12} is methyl.

25. A process according to any of claims 17 to 24 which is carried out using an oxidising agent selected from ozone in conjunction with hydrogen and a palladium-carbon catalyst, with zinc and acetic acid, with iodide and acetic acid, with dimethyl sulphide, with thiourea, with triphenylphosphine, with trimethylphosphite, or with pyridine or periodate, for instance in the presence of a catalyst such as osmium tetroxide, or potassium permanganate, sodium periodate with potassium permanganate catalyst,

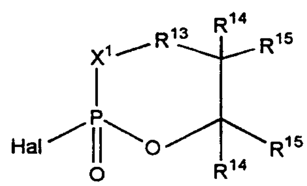
with ruthenium (III) chloride or ruthenium (VI) dioxide catalyst, (bi py)H_2CrOCl_5 and potassium permanganate and silica gel, preferably ozone with triphenylphosphine.

26. A process according to claim 24 including steps for the formation of the compound of the formula VIII by the reaction of a compound of the formula IX



IX

in which the groups R^3, R^4, R^6, R^9, R^{10}, R^{11} have the same meanings as in the compound of the formula VIII, X^1 and X^2 are as defined in the group of formula V is reacted of the compound with a phospholane reagent of the formula II



X

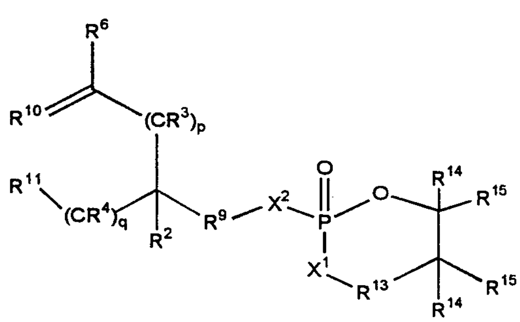
in which Hal is a halogen atom, preferably chlorine,

R^{13} is a bond or a group $C(R^{14})R^{15}$,

each group R^{14} is selected from hydrogen and C_{1-4}-alkyl groups;

each group R^{15} is selected from hydrogen and C_{1-4}-alkyl, or two groups R^{15} may form a C_{1-5}-alkylene group

to produce a phospholane intermediate of the formula XI



XI

in which all the groups have the same meanings as in the respective compounds of the general formulae IX and X, and in which the intermediate of the formula XI is subjected to a ring opening amination with an amine NR^{23}_3 reagent to produce the compound of the formula VIII.

27. Crosslinked proteinaceous materials produced by a process in which a zwitterionic crosslinker of the formula I

$$X_m (R^1) (CHO)_n \qquad \text{I}$$

in which X is a zwitterionic pendant group having an overall neutral charge,

R^1 is an organic group having n+m functionality,

m is at least 1 and

n is at least 2,

or a gem-diol, hemiacetal or acetal derivative thereof, is contacted in aqueous solution with a substrate having pendant primary amine groups, under conditions allowing reaction of the CHO groups of the compound of the formula I with the primary amine groups of the substrate.

28. A product according to claim 27 in which the substrate comprises protein, preferably comprising collagen.

INTERNATIONAL SEARCH REPORT

Interna' l Application No
PCT/GB 98/01646

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08H1/06 A61L27/00 C07F9/09 C07F9/117

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08H A61L C07F C08L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 06249 A (ALBRIGHT & WILSON LIMITED) 1 April 1993 ---	
A	US 4 544 638 A (ROBERT C. SIEGEL) 1 October 1985 ---	
A	US 5 328 939 A (TAMMY SMITH) 12 July 1994 ---	
A	EP 0 566 924 A (BASF) 27 October 1993 ---	
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 September 1998	21/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lensen, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 12, no. 143 (C-492), 30 April 1988 & JP 62 258390 A (ORIENTAL YEAST CO LTD), 10 November 1987 see abstract & DATABASE WPI Week 8708 Derwent Publications Ltd., London, GB; AN 353332 A see abstract -----	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/01646

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